© Military Pharmacy and Medicine • 2013 • VI – 1 • 19 – 32

Toxicology

Microbes indicatorts of cosmetic preservation efficiecy. Part III: *Candida albicans*

Jerzy Mierzejewski[1], Agnieszka Woźniak-Kosek[2], Jarosław Kosek[3,]

[1]Emeritus Professor at the Military Institute of Hygiene and Epidemiology, Pulawy; Professor at Kazimierz Pulawski Technical University of Radom, Faculty of Materials Science, Technology and Design, Chair of Chemistry, Radom, Poland.
[2]Departament of Influenza Virus Research, National Influenza Center; National Institute of Public Health-National Institute of Hygiene, Warsaw, Poland..
[3]Military Institute of Medicine, Otolaryngology Clinic, Warsaw, Poland.

Author's address:
Agnieszka Woźniak Kosek, Departament of Influenza Virus Research, National Influenza Center; National Institute of Public Health-National Institute of Hygiene; ul. Chocimska, 24, 00-791 Warsaw, Poland; phone: (+48) 22542 1274, e-mail: akosek@pzh.gov.pl.

Received: 2012.12.28 • **Accepted:** 2013.03.13 • **Published:** 2013.03.30

Summary:
Candida albicans is an indicator of the official evaluation of the effectiveness of cosmetics maintenance. In the paper *C. albicans* general characteristics, morphology and culture, diagnosis of infections caused by the yeast, the role in the environment, pathogenicity, sensitivity and resistance to antifungal medicaments and the current interest in the microorganism in microbiology cosmetics were discussed.
The paper is to strengthen the belief of producers and users of cosmetics to the validity of the selection of *C. albicans* as an indicator organism to assess the effectiveness of added preservatives.

Key words: *Candida albicans*, cosmetics, contamination, conservation, antifungal medicaments.

Introduction

In cosmetics, as well as in other products made from organic material, in addition to bacterial contamination, there is microscopic fungi contamination as well. The contamination sources can be varied, starting from materials containing hyphae of fungi, through production rooms with excessive humidity, apparatus and non-cleaned and non-disinfected equipment without regular cleaning and disinfection and finally hygienically wrong way of manufacturing, packaging and use of cosmetics. Lytic enzymes, in which fungi are particularly rich, while breaking the products influence on reduction of qualitative values and completely disqualify the utility cosmetic very often.

As it was explained in the previous parts (***MILITARY PHARMACY AND MEDICINE***: Volume V, No. 2, 2012 pp.32-41 and Volume V, No. 3, 2012 pp.17-30), manufacturers are required to determine the minimum, but effective concentrations of preservatives for each cosmetic product in order to prevent spoilage of them. These measures may be the same recipe ingredients (even one component) with both preserving and skin care properties. However, preservatives are often added to products. They need to work effectively

both antibacterial and antifungal, and the assessment of the effectiveness of their actions is performed with the so-called controlled contamination with microbial indicator.

This part of the paper contains information on indicator of fungal contamination, which is officially recognized as *Candida albicans* yeast. It belongs to the *Candida* genus brings together many species, 14 of which exhibits characteristics of pathogenicity. *C. albicans* is recognized as the most important pathogen in this group that causes opportunistic infections in humans called candidosis.

Candida albicans as commensals

C. albicans occurs mainly on the mucosal surface of the oral cavity, urogenital duct, vagina, and some areas of the skin, without causing infections in healthy people. Human mouth is extremely conducive environment for a variety of species of microbes and after birth it is inhabited by the kind of microflora very fast. The development of the microflora in the mouth promotes diverse area, favourable chemical and biological properties of the mucosa, a constant temperature (35-37°C), neutral pH and the presence of nutrients, vitamins and microelements. Yeast (*C. albicans*, *C. glabrata*, *C. tropicalis*) are among the dominant organisms as well. *C. albicans* is also mentioned as commensal of the upper respiratory tract, stomach, colon and large bowel, urogenital system, mainly vagina [1,2].

It was stated that there are differences in species of *Candida* in the production of alcohol (farnesol and tyrosol) being quorum sensing signalling molecules. These differences suggest different levels of invasiveness of the species. On the other hand, the production of the same molecule by different species indicates that they can influence each other and coordinate behaviour within a mixed population [3].

Commensal strains can be detected in about 50% of the human population, except that the *C. albicans* is the predominant (about 70%) representative of the *Candida* genus at various sites on the human body.

The ubiquity of microscopic fungi provide their detection even in a wide variety of finished cosmetic products to use. Fungi are generally responsible for the lowering of the quality of cosmetics and in exceptional cases, can cause infections especially in immunocompromised users. It speaks for the desirability of forming appropriate consumer awareness and the constant care of the quality of manufactured products. Proper use and storage of cosmetics should protect them from deterioration and the consequences of the use of contaminated products [4].

Predisposition to infections with *C. albicans*

As it was shown above, *C. albicans* colonization has specific places in the body where it can take the form of infectious. A few factors promote the formation of an infection: humidity, liquid acidic soap, topical corticosteroids [5] and a number of health predispositions, long-term treatment, and various medical treatments. These abilities include in particular: immunosuppression, steroid treatment, prolonged catheterisation, surgical treatment within the abdomen, treatment with antibiotics with a broad spectrum of activity, the perforations in the stomach and intestines, severe skin burns, mechanical ventilation, decreased kidney function, bone marrow transplantation, premature birth of a child with low weight and critically ill newborns, glycosuria, HIV infection.

In addition to endogenous infections rare cases of infections of external origin, caused by contaminated solutions and related materials catheterisation and transfusion were also described [6].

C. albicans virulence factors

C. albicans has its own mechanisms of invasion, in addition to resulting from host medical condition or treatments. These are:

- morphogenic change of yeast to filamentous form. This form enhances the adhesion and the ability to invade host cells by factors related to fillment including the adhesion molecules, the mycelium adhesion molecules, and the secreted hydrolytic enzymes.

Strains of *C. albicans* with more virulence factors and easily transfer from the yeast into the

pseudo and the true filamentous forms represent a particularly serious problem. These strains are capable of adhesion to the substrates, both biological and inert to the production and secretion of hydrolytic enzymes and biofilm formation [7].

Extracellular enzymes as virulence factors

C. albicans is a manufacturer of extracellular hydrolytic enzymes that beyond simple digesting food molecules, perform other functions. Among them there are three most significant extracellular hydrolytic enzymes: phospholipase, lipase and partially secreted protease apartyl (Saps).

Phospholipases are important determinants of pathogenicity. There are four types of secretory phospholipases A, B, C and D. Among them, phospholipase B contributes to damage of the host cell membrane by destroying phospholipid, fungi transfer and eventually leading to cell lysis.

Lipases are enzymes which catalyze both the hydrolysis and synthesis of triacylglicerole. *C. albicans* can produce at least 9 lipases that can hydrolyze the ester linkages are mono-, di-and triacylglicerole. Supporting the infectious process by lipase was confirmed in studies on the model of hematologically disseminated candidiasis in mice.
Lipase activity is considered as one of the major virulence factors of *C. albicans*.

Among the 19 various hydrolytic enzymes, studied in yeast-like fungi that cause mastitis in cows leucine arylamidase showed the highest activity [8] and asparyle proteases (asparyle proteases (Saps) are the most associated with the virulence of strains isolated from humans [6]. They belong to a group of cysteine and asparyle hydrolases, and metalloproteases [9].

C. albicans secretes apartyl proteases (Saps) representing a family of 10 related proteases [6]. The studies using the technique of immune-signing with gold reveal that Sap proteins are localized in the cell wall of *C. albicans*, both in the form of filamentous and yeast associated with virulence of *C. albicans*. In addition, proteases seem to have specialized functions preventing the antimicrobial activities of the infected host, e.g. Saps proteases degrade and inactivate the central human complement components C3b, C4b and C5 and block the effects of the risk of activated complement system. Sap genes showed a differential expression according to the morphological form of the fungus and the surrounding environment. Saps discloses as filamentous form is driven by the use of the polypeptide medium culture. It was found that the yeast form of the cells produce mainly Sap4, while filamentous cells produce mainly Saps 6.

However, so far there has not been complete knowledge of the Saps expression in various *C. albicans* pleomorphic forms.

In fact, there are no comparative studies conducted by different authors, using the same technique, model or the site of infection [6]. Regardless of these differences it is certain that the secretion of Saps asparyle protease components plays a key role in the pathogenesis of *C. albicans* infections [6].

Forms of *C. albicans* infections

Fungi of the *Candida* species are the most common pathogens among yeast-like fungi. Frequently it comes to skin infections within the skin folds where are the favourable conditions that include heat and moisture. Infections of the skin cause skin damage, injury rates, and feet and nasis damage, and chronic local application of corticosteroids and an increased concentration of carbon present in terms of occlusion help them [10].

C. albinans infections are divided into superficial and deep (systemic) [11.6].

Superficial infections are mainly mucosal and epidermal candidiasis. These include: oro pharyngeal candidiasis (oropharyngeal candidiasis — OPC).

OPC is the increased risk seen in smokers of tobacco or in patients with the following disorders: xerostomia, Sjogrens syndrome (SJS), a local cancer treatment preceded by mucosal injury, and patients with systemic treatment with steroids and antibiotics. In addition, OPC is one of the first clinical signs of HIV infection, and is recognized in over 95% of patients with AIDS. The main OPC lesions are alleged membrane

(called thrush), redness and inflammation of the mouth angles.

Cutaneous candidiasis – CC

Fungal infections of the skin concern the most often its superficial layer and appendages: nail and hair [10]. When growth of the skin microflora is inhibited (e.g. as a result of intensive antibiotic therapy), the epidermis is often infected with *C. albicans*. Excessive and undesirable long baths with products that contain detergents are not recommended as well [12].

Unusual skin infections take the form of deep fungal infection, including the deeper layers of skin coat and may develop in the case of immunological disorders.

Vulvo inguinal candidiasis (vulvovaginal candidiasis – VVC)

C. albicans is responsible for 85% cases of VVC for over 75% of all women. In addition, approximately 5-10% women have recurrent form. Unlike systemic candidiasis, characterized by the presence of *C. albicans* in a normally sterile body areas, VVC and recurrent forms attack the vaginal tissue where *C. albicans* can be normal commensals.

Chronic mucosal candidiasis (mucocuaneous candidiasis - CMC).

It is characterized by recurrent chronic infection of the oropharynx and esophagus with no tendency to systemic spread and increase in frequency of problems in the functioning of the endocrine glands.

C. albicans systemic infections

In addition to diseases of the skin and mucous membranes against fungal one distinguishes deep mycosis and organ mycosis, causing the formation of inflammatory processes in tissues, often necrotic. However, as the duration of the chronic inflammatory process starts and after some recovery time in the inflamed areas scar tissue may appear [13].

In the systemic infections *C. albicans* **can attack**:

1) respiratory system (acute primary pneumonia, secondary pneumonia, bronchitis, and aspiration pneumonia);

2) Urinary system (cystitis, pyelonephritis, infection of the implanted kidney);

3) Digestive system (from the described above oral candidiasis, to candidiasis of esophageal, stomach, colon, peritonitis, cholecystitis); Intestinal candidiasis include catarrhal inflammation, hemorrhagic-necrotic or changed membranous;

4) Central nervous system (inflammation caused by *C. albicans* penetrating the bloodstream during fungemia);

5) Osteoarticular system (inflammation of marrow and bone, muscle inflammation);

6) Inflammation of the eye and endocarditis is included in deep and organ candidiasis as well.

General candidiasis (sepsis symptoms are usually lighter than bacterial sepsis. It often accompanies of inflammation of the mouth and disorders of the digestive tract) [11].

Hospital infections of *C. albicans*

In the United States and Europe in recent years there has been a significant increase in fungal hospital infections. About 30 species of fungi that are the perpetrators of infections are mentioned. The share of fungal pathogens compared to other etiological factors have increased in recent years twice [10]. *Candida*, as a factor in hospital infections occupy fourth place after S. aureus infections, coagulase negative staphylococcus and P. aeruginosa [10]. As elsewhere, here *C. albicans* is an opportunistic pathogen manifested as pathogenicity after the removal of the normal bacterial flora by chemotherapy [11]. Thus they are endogenous infection, but in the case of *C. albicans* infection in neonates, these are exogenous infection as a result from poor hygiene of the environment.

In hospital infections are a serious problem resulting from transplantation procedures. Fungal

infections generally occur shortly after the treatment, as a result of intensive administration of chemotherapeutics. It was found that liver transplants pose a greater risk of fungal infections than kidney or heart transplants.

In bone marrow transplant recipients prolonged neutropenia is the most important factor disposing to the formation of fungal infections. [11].

An effective tool for the treatment of hospital infections, including fungal infections, is a rapid diagnostics using the method of real-time PCR [8].

Participation of *C. albicans* in the pathogenesis of other diseases

Studies involved several thousands of patients with non-specific gastrointestinal symptoms revealed that the presence of fungi was in 61.5% of stool samples and 70.9% of these *C. albicans* was found. In 20.8% of cases there was a breach of intestinal microbial balance. The study of isolates drug resistance showed lower than the normal sensitivity to azole derivatives, amphotericin B and 5-fluorocytosine [14].

Other clinical reports indicate that *C. albicans* can among others cause infection in patients with systemic lupus erythematosus [11]. Easy locating of the *C. albicans* in cracks in corners of lips is a known phenomenon. Apart from bacterial superinfections, yeast superinfections as off-white macerated skin detachment can be found there [15].

C. albicans in the pathogenesis of allergic diseases

Hypersensitivity to fungi of *Candida* kind, causing superficial fungal infections of the skin and mucous membranes, can lead to a severe urticaria.

Among type II hypersensitivity reaction to fungal antigens, sporadic cases of allergy to mannan (polysaccharide cell walls of fungi, among others of *Candida* species were described. Mannan is a major allergen in patients with atopic dermatitis. Diseases related to hypersensitivity to fungal allergens include asthma, bronchial asthma, allergic rhinitis, allergic sinus mucosal inflammation, allergic conjunctivitis, atopic dermatitis and urticaria. In one study, the authors report that in 57% of patients with asthma the *Candida* fungus allergen derivatives were found. If you are allergic, it is proposed to carry out analysis in the direction of the fungal infection in order to reduce the severity of the treatment of asthma include anti-infective Trychophyton fungi, *Candida* [10].

Epidemiology and fighting *C. albicans* infections

The percentage of fungal infections caused by *C. albicans* is relatively high in the USA. In Europe, the number of infections in humans is also increasing. The appearance of the most dangerous forms of candidiasis, candidemia in intensive care units, is particularly disturbing. Studies in neonatal intensive care units in the United States and France have shown that *C. albicans* was the most common species responsible for invasive candidate infections in neonates. Disseminated and invasive candidiasis seem to be very similar in the U.S. and Europe, and are estimated to be 49 to 55% of all candidiasis. Among the predisposing factors for candidemia and disseminated candidiasis are the following: cancer (26%), surgery within the abdomen (14%), diabetes (13%) and AIDS (10%). According to statistical data of the etiology of systemic fungal infections of *C. albicans* formed a serious medical worldwide problem. The high incidence of these infections, as well as the high mortality of patients with immunosuppression increase the interest in the study of the virulence factors of *C. albicans*i and the appropriate candidiasis treatment strategy [6].

C. albicans infection sources can also be found in the animal world. Even the outer shell of free-living animals the presence of numerous opportunistic fungi, including *Candida* was detected [16]. It was reported that from the samples taken from the dog with demedocosis *C. albicans* was isolated without bacterial growth. This strain was sensitive to flucytosin, amphotericin B, and nystacine and only moderately sensitive to fluconazole and ketonazole. Oral treatment with Amitraksem (an antiparasitic), and in conjunction with ketonazole mikonazole ointment resulted in complete cure within 9 weeks. Demedocosis infection and colonization of *C. albicans*

on lesions are likely to lead to a synergistic effect of the parasite on the fungus. Isolation of *C. albicans* from lesions and positive reaction of a sick animal to antifungal treatment confirmed the role of the yeast in the described infection [17].

Diagnosis of *C. albicans*

Isolation and identification of *C. albicans* in clinical laboratory

Clinical material should be obtained directly from the place where the disease process takes place and tested as soon as possible. From the point of view of cosmetologists skin, nails and hair are important areas. Skin scrapings should be taken with a scalpel in a few places with the most recent changes to the banks. The vesicles or pustules, purulent samples are taken with a swab moistened with saline. Similarly, samples are taken from the mucous membranes.

The affected several hairs are collected using tweezers. Samples are taken from under the changed nail.

Skin, nails and hair are opaque. Prior to microscopic examination of samples a solution of 10-30% NaOH or KOH (or 10% of sodium sulphide and laluryl sodium sulphate) is to be addend. Such radiolucent preparations were tested under low and high magnification. According to Grama, Giemsa, etc. the formulations can also be dyed with blue methylene. On the medium surface Sabouraud grows in the form of damp, shiny, grey-white colonies. The microscope slides are shown round or oval cells, which can be in the process of budding (yeast phase, the phase of Y — yeast), or create pseudohypha (mycelial phase - M).

Clinical material is placed on Sabouraud solid surface, where the macroscopic appearance of colonies is estimated. They are damp, shiny, grey-white.

The sexual form of *C. albicans* has recently been described. It shows features of basidia. It comes from *C. albicans* chlamydospore with double amount of DNA in a cell which is in the course of budding [11].

Further discussion of research techniques of cosmetic raw materials and the direction of *C. albicans* are in the official rules [34,35,36] and normative acts discussed further below.

In addition to *Candida* in clinical material there has been a type of Rhodotorula, Pityrosporum, Trichosporon yeast. Rhodotorula species are found in healthy people, but also in the materials from patients with respiratory infections. Pityrosporum yeasts are visible in the preparation of material from patients as a round or oval cells.

Among described several species of Trichosporon only Trichosporon cutaneum is pathogenic for humans. It causes hair fungus (white piedra).

Serological diagnosis of *C. albicans*

Over 80% of infections caused by budding yeast are attributed to *C. albicans*. Antigen selection of a large difficulty in serological diagnosis. In addition, some C. albcans antigens were detected in other species, therefore serological cross-reactions may occur [11].

The most often used group is glycoprotein antigens constituting the cell wall with active polysaccharide ingredient (mentioned mannan is the most commonly used antigen) and intracellular proteins. The demonstration of mannan is important to differentiate the form of candidiasis. Several tests are used simultaneously to confirm the causative agent of infection.

Serological methods are sensitive for the detection of IgM antibodies to polysaccharide antigens. Various modifications of hemagglutination reaction are used herein with polysaccharide extracts of *C. albicans*. In healthy patients 1:20-1:40IgA class antibodies are often found, while in ill patients above 1:160. Later in the infection, in similar sensitive hemagglutination reactions towards polysaccharide antigens, IgG antibodies are detected.

Antibodies to protein antigens (intracellular) are detected in precipitation, immunodiffusion, immunoelectrophoresis tests. In immunosuppressed patients, especially in the systemic candidiasis and chronic mucosal candidiasis antibodies of *C. albicans* antigens are detected, both

protein and polysaccharide. Latex and ELISA tests are applied here [11].

Methodology for susceptibility determining

As with bacterial infections, fungal infections knowledge of the pathogenic fungus sensitivity to chemotherapeutic agents is the basis for rational treatment. To evaluate the susceptibility quantitative and qualitative methods are used.

Quantity methods

They are used to determine the minimum fungicidal concentration - MFC. Dilution methods in a solid or liquid surface are applies for this purpose. Dilution micromethod is still the reference method (MIC/ in mg/l).

Another method is applied to determine the chemotherapeutic concentration causing 50% inhibition (IC50) or 90-99,9% (IC99, 99%) of yeast cells in liquid culture [11].

Diffusion cylinder method in agar is another embodiment. MIC value is calculated from the curve of the relationship between a zone of growth inhibition around the wells in the agar and the logarithm of medicament concentration.

Quality methods

These are diffusion method using flimsy discs soaked with appropriate antifungal medicament. Practically they are used for routine susceptibility testing for flucytosine and some imidazole.

For polienyle antibiotics diffusion cylinder method is applied the most often. It is recommended to use a synthetic substrate of specified composition as YNB-agar (Yest nitrogen base + 2% glucose, 1.7% agar), SAAF (synthetic amino - acid fungal medium), MVA (yeast morphology agar), and others. Note that the popular Sabouraud surface used to determine the susceptibility, may exhibit antagonistic effects of synthetic antifungal compounds.

The result for susceptibility of yeasts to chemotherapeutics is achieved after 48 hours of smearing in 37°C [11].

Body defence against infections

In the development of candidiasis transition from health to disease occurs when there is interaction between the fungus and the mucosal membrane (or skin of the host). Resistance to *C. albicans* is involved with both innate and acquired immune response mechanisms. Status of the immune system of the infected person is the main barrier inhibiting the transition of *C. albicans* commensals to the form of the pathogen. Likewise, in preventing the spread of pathogens from the site of infection plays an important role in defence mechanisms of mucosal/ or the skin of the host

The defence against infection is involved with many types of innate immune cells: neutrophils, monocytes, macrophages, NK cells, dendritic cells, T cells, epithelial cells and keratinocytes mucosa.

In case of the system infections release of interferons (IFs) and lymphotoxin (LTA) from Th1 cells is responsible for activating the antifungal properties of neutrophils and macrophages in the deep tissues. IL17 and IL22 release from the specific cells causes accumulation and activation of neutrophils to the elimination of mucosal infection. It should be noted that in the reduction of infection in the body various other commensals in the oral cavity, GI tract and vagina play an important role.

Specific prevention

Skin infections are usually self-limiting and treatment is associated with a state of limited immunity to reinfection. This resistance probably depends on the IV type answer (delayed) to the fungal antigens. Cell-mediated immunity seems to be important in other fungal infections, since it can be moved by means of sensitized T cells. It is supposed that TH cells release cytokines that activate macrophages to destroy fungi.

As it was already emphasized, immune deficiency caused by immunosuppressive medicaments

or destruction of normal bacterial microflora by antibiotics may be the reasons for the invasion of *C. albicans*. *C. albicans* infection, often seen in congenital or acquired immunodeficiency, suggest that only the mechanisms of proper resistance are able to eliminate or control the growth of fungi [18].

The above explanations justify the lack of prospects for the production of an effective vaccine against candidiasis, although it was already in 2002 that American sources signalled candidiasis vaccine was at the stage of research, development and preclinical studies [19].

Treatment of infections and medicaments sensitivity of *C. albicans*

Antifungal chemotherapeutics are represented by the different groups with different properties and different pharmacological action time [11]. Traditional antifungal agents, particularly fungal surface, were simple chemical compounds (salts of mercury, chlorine, and iodine). Currently these compounds are sometimes used for the treatment of fungal infections of the skin (ointments, powders, suspensions). Moreover, there are preparations containing phenol derivatives, salicylic acid, sulphides, sulpfonates, hydroxycholine derivatives, invert soaps. In the treatment of superficial mycoses solutions of the dyes are also used, such as bitterness violets and ointments containing it [20]. Using pharmaceuticals for external use their impact on the behaviour of antimicrobial properties of the skin needs to be analyzed. Their adverse effects was demonstrated in the study of the effectiveness of anti-mite preparations: oxalic acid and amitrax and formaldehyde. They reduce antimicrobial activity (e.g. against *C. albicans*) of the surface of the skin. Upon activation of these preparations the activity was decreased or disappeared for a period of 4 weeks [20].

As indicated, in the pathogenesis of candidiasis the first colonization step is the adhesion. Quaternary ammonium compounds seem to meet these expectations by showing inhibition of the adhesion properties. Among alanine and glycine-derivates containing ten-coal alkyl chains are those which do not show any mutagenic while effectively combating biofilms and are inhibitors of H +-ATP yeast membrane [21]. There is a growing interest in ksantone derivatives, which also has a high antifungal activity. The studies on the effect of ksantone derivatives on dimorphism of *C. albicans* cells showed that the most active compounds contain chlorine and prenyl derivatives in the molecule. These compounds significantly reduce or completely deprive the fungal cells the ability to pass in a virulent filamentary form [23]. Much attention is given to research activity of essential oils against yeasts, among others, *C. albinas* [11]. Inhibitory concentration of ethereal oils results in 2-4 fold increase in sensitivity of *C. albicans* to factors penetrating cell guards. Similarly, tolerance to oxidative stress is significantly reduced. For this reason opportunity to improve the effectiveness of antifungal specifics by associating them with oils activities emerges. Such a strategy can be proposed at least for the topical treatment of chronic infections of C. albonas [24]. Positive results were obtained also for volatile fractions of ethereal oils against fungal biofilm infections causing chronic local infections. Because of the potential cytotoxic and irritant effects of medicament oils in a cream, ointment or talc the effects of the volatile fraction appears to be a good treatment [25].

C. albicans as commensals in the oral cavity poses a threat in the increasingly popular dental implant. Such treatment requires appropriate antiseptic preparation of the oral. Many patients have a prosthesis which predispose to oral colonization by yeast-like fungi capable of making biofilm as a danger to the success of the surgery and maintenance of the implant. For the best selection of mouthwash fluids the activity of selected solutions was assessed: 0.1% chlorhexidine digluconate, tincture of arnica, potentilla rhizome extract, plantain leaf, marigold basket and the arnica basket. A solution of 0.1% chlorhexidine digluconate demonstrated fungicidal activity with respect to all strains of *C. albicans*. Tinctures and extracts inhibited the growth of strains or did not show antifungal activity in varying degrees. Thus, in order to decrease the amount *C. albicans* before implantation preparations of chlorhexidine digluconate can be used, and plant preparations at the recommended concentrations are not suitable fungicidal activity [26].

As it was already discussed above numerous enzymes are largely responsible for the virulence of *C. albicans*. Therefore, the effect of ethereal oils was tested: clove, geranium, melissinic and cytroneol on the activity of phospholipases, proteases and hemolysin produced by *C. albicans*. The validity of the assumption was shown that the etherreal oils used in lower subinhibition concentrations decrease the activity of several hydrolytic enzymes performing an important role in the pathogenesis of *C. albicans* infection [27].

There are efforts to take advantage of oriental medicine, often popular for the treatment of fungal infections. The attention is paid on the antifungal activity of Raoultella ornithinolica bacteria that lives in Dendrobanea veneta earthworm gut wall. Glyco-protein complex purified from fluid postcultivated from the bacteria in preliminary experiments showed antifungal activity against *C. albicans* [28].

Antifungal treatment with chemotherapeutic agents

The final stage of mycological diagnosis, in addition to the identification of fungi and to determine the amount of clinical material, is the assessment of the sensitivity to antifungal chemotherapeutics [29].

The main groups of chemotherapeutic agents include antibiotics, antifungal and synthetic chemotherapeutic agents from the group of imidazoles. Pyridone derivatives, morpholine and allylamine were introduced into candidiasis medical treatment [11].

Only a few antifungal antibiotics with hundreds had therapeutic use. They can be divided into two groups: polyene and non-polyene macrolide antibiotics.

Polyene antibiotics

These are products of metabolism of actinomycetes (Streptomyces). They are effective against fungi, including opportunistic yeasts. The ready-made specifics of the final group include popular nystatin and polifigmine, on which are the most strains of *Candida* are sensitive. Similarly, natamycin (pimarycin) known as primafucin acts on the yeast.

Another antibiotic amphotericin B (preparations: Amphonoronal and Fungilin) is considered to be the most important antifungal medicament. In combination with dioxycholane sodium it is produced as Fungizone preparation for the treatment of general and systemic fungal infections.

Non-polyene antibiotics

These are a variety of compounds having common antifungal feature. Medical use was found among others in Griseofulvin and Aktydion (cycylohexide). The latter is added to the substrate as a selective agent.

Griseofulvin (prep. Commercial Gricyn, Gryfulwin, Grisovin, Gryseofulvin forte) is toxic and photosensitising.

Imidazoles

Among others metronizadole is a synthetic compound involved in a group of azoles (imidazoles, triazoles or trizoles). Clotrimazole and miconazole show activity against yeasts. Note that the activity of these compounds depends on the type of fungus, and even strains within a species. They are highly effective against *C. albicans*, but are inactive against other species of this type (C. kruzei and *C. tropicalis*) [11]. Other authors have confirmed that the spectrum of activity of the imidazole derivatives is varied to different *Candida* species [30]. However, different chlorides of imidazole derivatives may have high activity against *C. albicans* [31]. Enikonazol belonged to the group of imidazoles as antifungal was applied to combating fungi on living environment of poultry and other animals. Enikazol proved to be an effective agent for a broad antifungal activity against opportunistic fungi, but also against yeasts, among others *Candida* [32]. Imidazoacridines appeared to be photosensitising compounds [33].

Flucytosine

It is a synthetic compound known as 5-fluorocytosine (5-FC) with high activity against yeasts. It influences on ribonucleic acid by blocking the synthesis of protein.

With the synergistic effect with amphotercin B it is sometimes the only medicament with systemic mycoses and sepsis [11].

Candida albicans in clinical and microbiological purity assessment of cosmetics according to legal requirements

Microbiological requirements for cosmetics are specifically defined in the law, where *C. albicans* is considered as the only eukaryotic test organism. Cosmetics Act of 30 March 2001 sets out a number of requirements that must be met by cosmetics manufacturer. One of the articles of this law refers to the microbiological purity [34]. However, the Act does not provide any specific guidelines for microbiological control and acceptance criteria, testing methods. These guidelines can be found in the Regulation of the Minister of Health of 23 December 2002 on the definition of cosmetics sampling procedures and laboratory testing procedures [35]. While cosmetics manufacturers are required to produce cosmetics that meet the requirements of this regulation, they may use their own, more stringent criteria and testing methods. The regulation describes how to test the preservation of products, handling of raw materials and semi-finished and finished products, provides the requirements of the microbiological purity of cosmetics and research methodology. Maintenance effectiveness of cosmetic products protecting against fungal contamination is being investigated in the stress testing of *C. albicans*. The test load controls systems preservative efficacy against *C. albicans*, based on pharmacopoeia requirements and their own experience of laboratory control. As in the case of products. This regulation indicates the need to study the same materials and intermediates in the direction of *C. albicans* contamination as well. In terms of microbiological requirements cosmetics are divided into two categories:

Category I: cosmetics intended for children, and around the eyes.
Category II: other cosmetics.

Microbiological criteria include establishing, inter alia, the total number of *Candida* albicans.

Proceeding

a) Preparation of the homogenate
Weigh 10 g of the product into a sterile container (in the case of a small weight 1g can be weighed). After weighing the sample dilute 1:10 with the dilution fluid. After dilution, the sample is shaken until complete homogenisation. In case of the anticipated growth of microorganisms and prepare dilution of 10-2 and further.

b) Detection of the presence of C. albicans in 0.1 g of product.
Homogenate diluted samples in a volume of 0.1 ml are plated using the "pitch method" on the solid Sabouraud surface. *C. albicans* grows in the form of white or beige colonies. In the presence of these colonies further identification should be done by plating on chromogenic substrate specific for *C. albicans*.

No characteristic colony appears to be interpreted as the absence of *C. albicans* in 0.1 g of the product.

Specific requirements for cosmetics are as follows:

CATEGORY I. Care for children and the eye area:

Total number of *C. albicans*, as well as testing bacteria can not exceed 100 cfu/g or ml (CFU — colony forming units).

CATEGORY II. Ather cosmetics

The total number does not exceed 1000 cfu/g or ml.

CATEGORY III. Regulation of the European Parliament and of the Council 1223/2009/WE of 30 November 2009 on cosmetic [36].

It shall apply from 11 July 2013. It does not contain specific research methods or define microbiological requirements, but refers to harmonized standards:

In accordance with Article. 12: "research methods must be in accordance with the relevant harmonized standards, the references of which have been published in the Official Journal of the European Union". Harmonised standards are developed by ISO, as generally applicable in the Member States. The list of harmonized standards

provide the standard: PN EN ISO 18416:2009 for the detection of *C. albicans*.

Current interest in *C. albicans* in cosmetic microbiology

As it was already stressed, *C. albicans* is the only eukaryotic organism officially used to evaluate microbial contamination of raw materials and cosmetic products, as well as the efficacy of preservatives added to cosmetics. For this reason, the characteristics of the organism must be taken into account in any study on this subject. The presented literature review points out the role of asparyle proteases in the pathogenesis of *C. albicans* infections [6], the assessment of materials and substances filled with cyclodextrins and antiseptic substances that may have a use in cosmetics [37].

Continuing interest in the use of essential oils as ingredients of cosmetics is maintained [7]. Sensitivity of *C. albicans* to 12 different oils was tested and showed that most of them inhibit the growth of microorganisms, which indicates the possibility of their use as active ingredients of cosmetics used for example for oral hygiene. However, further in vitro studies should be conducted to confirm the effectiveness of antiseptic and low toxicity [38]. Much attention was paid to the action of ethereal oils against *C. albicans* alongside various species of dermatophytes and filamentous fungi capable of creating biofilms. For example, clove oil work in the volatile phase after 4 hours of exposure reduces *C. albicans* biomass by 1.33%. The concentration of geranium oil 1/2MIC against C. albcans caused the decrease in this concentration of added fluconazole with 12 mg/l to 0.064 mg/l. [7]. In a study of a synergistic antifungal medicines and the effect of ethereal oils several times lower MIC antimycotics values was obtained [7]. Similarly, flucanozole activity against clinical strains of C. albians with the combined effect of Melaleuca alternifolia tea tree oil increased [39]. It is proposed to evaluate the effectiveness of essential synergistic with fluconazole and voriconazole by MIC determination using test strips [40].

A new proposal concerning the way of preparing dressing materials which could be used in cosmetics, is filling dressings and antimicrobial substances with cyclodextrins. Cyclodextrins are natural cyclic oligosaccharides produced during the enzymatic degradation of starch by a bacterium of Bacillus genus. For antimicrobial substances, such as iodine, polihexamid and chlorhexidine acetate, placing in the cyclodextrins gives better compatibility with the skin, higher anti-microorganisms activity and greater durability in storage. Fabric with beta-cyclodextrin with polihexamid showed strong antifungal activity and complete growth inhibiting of *C. albicans* [4].

Summary

This paper is the third part of the monograph discussion of microorganisms contained in the of official rules for the microbiological testing of raw materials and cosmetic products. *C. albicans* is one of many microscopic fungi contaminating cosmetics. The paper presents its ability to cause a variety of opportunistic diseases and ubiquity as commensals in the human environment. Superficial skin disease and mucous membranes diseases closer to cosmetology were widely discussed.

The opportunistic ability of *C. albicans* not only to self-induce various diseases, but the pathogenesis of complications complicating pathogenesis and hindering the treatment of other diseases were shown. Research methodology and ease stress tests should encourage manufacturers of cosmetics to reach for verification of the effectiveness of maintenance and microbiological safety of cosmetic products and thus ensure the safety of their everyday use as much as possible. This study, because of monographic approach has didactic features and can be used by teachers in the preparation of lectures and exercises in microbiology, cosmetics and students writing theses as a comprehensive source of references.

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